

# **Premature ovarian failure in young females due to cancer therapy; challenges and options in terms of fertility preservation.**

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## **Summary**

### **Background**

It has become more and more important to address the topic of fertility preservation in young females due to drastic improvement in treatment of childhood cancer. About 75-80% of all children and adolescents recovered from cancer live more than five years after the time of diagnosis. There are many options for fertility preserving methods for adult females, but only cryopreservation of ovarian tissue is an option for females who have not reached puberty. The procedure of cryopreservation is still on an experimental stage, but a few live births are registered using this technique.

The aim of this literature study is to create an overview of common malignant diseases in children and adolescents up to 15 years of age, treatment of the diseases and adequate strategies to preserve fertility in these patients. The study also points out the different challenges in the area and where there is need of improvement concerning the different procedures.

### **Methods and material**

The study is based on relevant literature on the subject using PubMed databases, oncolex, Nordic Society for Pediatric Hematology and Oncology (NOPHO), The Norwegian Cancer Register, other relevant literature published and Google searches.

### **Results/Future directions**

Several countries, Norway included, offer fertility preservation to female patients in risk of premature ovarian failure due to cancer treatment which usually has devastating effects on ovarian tissue. The use of alkylating chemotherapeutic agents and radiation therapy in treatment of cancer reduces the number of follicles in the ovaries and cause premature ovarian failure, which is a sign of reduced fertility. The importance of knowing that cancer itself

affects fertility, should also be evaluated in terms of fertility preserving treatment. Today, the only option for fertility preservation in prepubertal females is cryopreservation of ovarian tissue. However, there are still several factors to elucidate concerning this procedure, for example whether a whole ovary – or just cortical strips with follicles should be preserved. The risk of reintroducing malignant cells should also be further elucidated. Nowadays, histological examinations as well as immunohistochemistry analyses are used to clear ovary tissue for retransplantation. However, studies emphasize that histological examination alone is inadequate to clear tissue for malignant cells. Studies using quantitative reverse-transcribed polymerase chain reaction (RT-PCR) could in the future be a solution to this problem. We also know that the follicle pool diminishes to about half the original size during the freezing procedure, which should be improved. The use of *in vitro* maturation of follicles is also a possibility for female fertility preservation in the future, but more studies are required before the procedure can be implemented. Studies point out the possibility of affecting the locally produced factors which mediate primordial follicle development in the ovaries as a way of preventing premature ovarian failure.

Finally, oncologists and other physicians responsible for cancer treatment in children and adolescents should have the proper training to adequately address the question of fertility preservation.

## **Introduction**

Childhood malignancies contribute to approximately 1% of the overall cancer incidence. From 2004 to 2008, 734 cases of cancer in children were registered in Norway, involving 404 males and 330 females. The restoration of female fertility has become more and more important with more advanced treatment for cancer and longtime survival. About 75 - 80 % of all children and adolescents with manifested cancer survive due to adequate treatment and live more than five years after the time of diagnosis.<sup>1</sup> Due to this generally high survival rate for childhood cancer, we are forced to further develop good techniques to ensure fertility in these patients.

### **Female reproduction system.**

The process which comprises this exquisite development of female gametes to mature oocytes, begins in utero and extends throughout life until menopause.<sup>2</sup> Around gestational week 18, the oocytes start to assemble in the female ovary. This process continues into the third trimester.<sup>3</sup> The follicle assembly process generates the production of primordial follicles, which constitutes the female reproduction capacity because no more follicles can be made.<sup>2</sup> The primordial follicle consists of the oocyte with a surrounding layer of squamous granulosa cells. The primordial follicle development also includes the transition from primordial- to primary follicle. From this point on the follicle will continue developing until the oocyte is ovulated, or it will undergo atresia and regress.<sup>4</sup> During female reproductive age several primordial follicles develop to primary follicles each menstrual period. From this time on the follicle pool gradually diminish, and finally when depleted - lead to the end of reproductive age and the female enters menopause. The transition from primordial- to primary follicle as seen in Fig. 1., is controlled by locally produced inhibitory and stimulating paracrine and autocrine growth factors such as kit ligand (KL), basic fibroblast growth factor (bFGF), leukaemia inhibitory factor (LIF), bone morphogenic proteins (BMP's), keratinocyte growth factor (KGF), basic fibroblast growth factor (bFGF), anti-müllerian hormone (AMH) and stromal-derived factor 1.<sup>4</sup> Knowing the factors controlling the transition from primordial- to primary follicle transition opens doors for better treatment for females with conditions causing premature ovarian failure. Premature ovarian failure is characterized by amenorrhea, raised FSH and low estrogen levels. The ability to inhibit primordial follicle development at one point, and increase the development at another point, could also be crucial for fertility in

females who are subfertile for various reasons and/or at risk of reaching menopause prematurely.

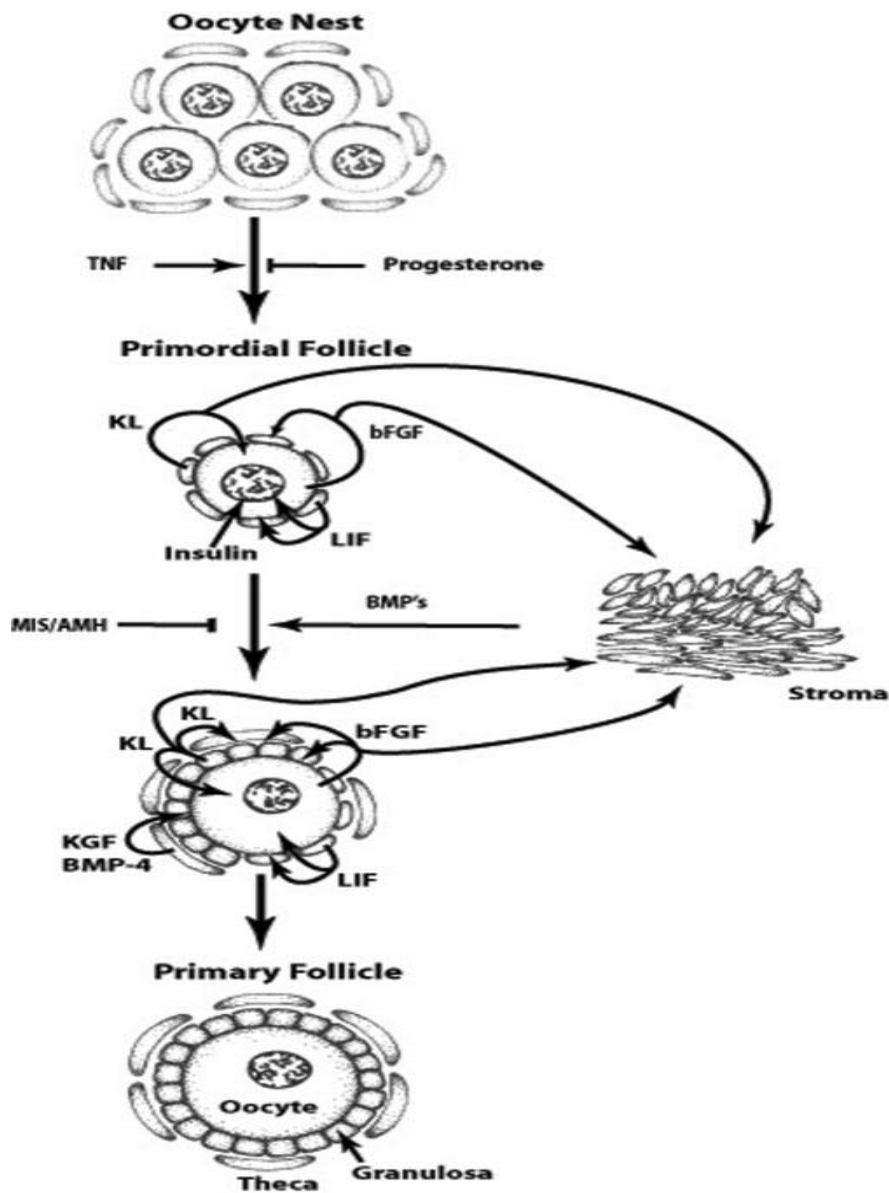


Figure 1 Schematic representation of the proposed cellular interactions in primordial follicle development. The follicle structures involving the oocyte, granulosa cells are shown. Cell-cell interactions are mediated by tumour necrosis factor alpha ( $\text{TNF } \alpha$ ), kit ligand (KL), basic fibroblast growth factor (bFGF), leukemia inhibitory factor (LIF), bone morphogenic protein-4 (BMP-4), keratinocyte growth factor (KGF), insulin and anti-Müllerian hormone (AMH).<sup>5</sup>

### Pharmacological treatment of cancer and effect on gonadal function

Cancer cells are characterized by a reduction in velocity of apoptosis compared to normal cells. They also have the ability to enhance mitosis, and thereby rate of growth. Their growth-pattern and cell differentiation are different compared to normal cells. Still, their basic cell characteristics are quite similar to each other. This similarity is a problem during treatment of cancer, because the treatment is not specified towards cancer cells.

There is great variety in terms of cell toxicity and ovarian damage between pharmacological options in treatment of cancer. It is also known that the risk of premature ovarian failure in terms of cytostatic treatment correlates to age. The density of follicles in ovaries in young girls is much higher compared to density of follicles in ovaries from older women. The difference makes young girls less vulnerable to cytostatic treatment than older women. The toxicity is also closely connected to doses. As the dose of treatment increases, the toxicity also increases. Scientific studies comparing female cancer patients treated with alkylating agents with females not receiving any kind of treatment support the fact that treatment with alkylating agents significantly increases the risk for premature ovarian failure.<sup>6</sup> Treatment with chemotherapeutic agents accelerates the reduction of the primordial follicle pool, induces ovarian atrophy and further a destruction of vascularisation of the ovary which leads to ovarian damage and risk of premature ovarian failure.<sup>7</sup>

Follicular apoptosis and cortical fibrosis of ovaries are also assumed effects related to treatment with chemotherapy. Studies with electron microscopy have demonstrated destruction of primordial follicles in presence of chemotherapeutic agents.<sup>8</sup> *In vitro* studies with primordial follicles and chemotherapeutic agents reveal accelerated apoptosis of the former. Nevertheless, this is still to be elucidated *in vivo*. The “burn out” hypothesis emphasizes lack of a locally produced factor, anti-müllerian hormone (AMH), as the ultimate cause of accelerated reduced follicular pool in patients treated with chemotherapy. Treatment with chemotherapy damages and destroys growing follicles which lead to reduced production of AMH.<sup>8</sup> The inhibitory effect of AMH on the locally produced stimulatory factors such as KL, KGF and bFGF drops, and more follicles than usual start to mature.

Alkylating chemotherapeutic agents such as cyclophosphamide, chlorambucil, melphalan, busulfan, nitrogen mustard and procarbazine are rated as high risk agents for developing premature ovarian failure. Furthermore, platinum derivatives like cisplatin, cytotoxic antibiotics like doxorubicin and taxanes are classified as intermediate risk agents for developing ovarian failure. Chemotherapeutic agents related to low or no risk of developing ovarian failure are antimetabolites like methotrexate and 5-fluorouracil, plant alkaloids like vincristine, cytotoxic antibiotic such as bleomycin and actinomycin D.<sup>8</sup>

However, treatment of malignant diseases usually involves a combination of drugs which makes it essential to evaluate fertility outcome in terms of that specific treatment. Several animal studies have already verified the effect of different drug regimes on ovary function. Treatment with alkylating agents in general has devastating effects on ovary function and the risk of developing premature ovarian failure is high.

#### Radiotherapy and effect on gonadal function and fertility

As with cytostatics, the effect of radiation towards the ovary in concordance to premature ovarian failure is correlated to dose of treatment and age of the female. Studies estimate that total radiation above 20 Gy distributed over six weeks, represents considerable risk for developing premature ovarian failure.<sup>6</sup> Young patients receiving total body radiation and treatment with alkylating agents due to planned bone marrow transplantation, are in high risk of developing premature ovarian failure. Most females who get this type of treatment do not enter puberty or puberty is not completed after treatment.<sup>6</sup> Despite efforts to try to protect the ovaries in terms of abdominal radiation, the protection is limited when the radiation field overlaps with the ovaries. Studies reveal up to 90 % risk of ovarian failure due to total body radiation, and as much as 97 % after abdominal radiation.<sup>8</sup> Studies reveal that about half of the follicles which are in a resting state in the ovaries are destructed when exposed for radiation doses of 2 Gy.

It is also important to consider the effect of radiation towards the uterus in terms of fertility. Radiation towards the uterus reduces the size of the organ, makes it less elastic, and therefore enhances the risk of spontaneous abortion and premature birth. It also enhances the risk of miscarriage as well as the risk of premature delivery.

The risk of affecting fertility is also an important consideration when young patients undergo cranial radiation to treat malignant disease. Cranial radiation may affect the hypothalamic pituitary axis, and thereby result in early onset of puberty with precocious menarche. In situations of cranio-spinal radiation, menarche can either be precocious or delayed.<sup>6</sup>

#### Methods for female fertility preservation

Options for female fertility preservation during treatment for cancer with chemotherapy and / or radiation today, consist of pharmacological treatment with GnRH-a, transpositions of ovaries in cases of abdominal radiation, cryopreservation of oocytes, cryopreservation of embryo and cryopreservation of ovaries.<sup>6</sup>

The pharmacological treatment with GnRH-agonist affects hormonal feedback mechanisms in the female body and leads to a hypoestrogenic state.<sup>6</sup> One assumes that GnRH treatment reduces velocity of follicle depletion during chemotherapy, due to reduced perfusion of the female reproductive system.<sup>6</sup> From about three months old until the time of menarche, the gonadotropin releasing hormone secretion from the hypothalamus is absent. Therefore, treatment with GnRH-agonist is not an alternative for prepubertal girls.

Transposition of the ovaries is a procedure of fertility preservation introduced already sixty years ago, and it is also known as oophoropexy. The procedure is characterized by relocating the ovary tissue out of the field of radiation, and thereby prevent ovarian failure.<sup>6</sup>

The oocyte cryopreservation also encompasses females in reproductive age, and consists of harvesting mature oocytes from the ovaries with subsequent cryopreservation. Regarding harvesting of mature oocytes, the technique involves ovarian stimulation for 4-6 weeks in advance to mature oocytes before the procedure, and thereafter use of transvaginal probe for collecting oocytes.<sup>9</sup> However, ovarian stimulation delays cancer treatment. Therefore this process excludes patients who are dependent of constant treatment and also young girls due to the procedure of using a transvaginal probe for collecting oocytes. This technique exclusively encompasses females who have entered puberty which is essential for oocyte development.

One option which probably will be available in the future is *in vitro* maturation of oocytes. The procedure does not require oocytes to be mature before collection, and is therefore independent of menstrual cycle. It is also possible to collect immature oocytes in

relation to cryopreservation of ovarian tissue. By bypassing the use of transvaginal probe for collecting oocytes, the procedure can be an option for younger girls and adolescents. This procedure is still to be elucidated, but in the future it probably will be one alternative for women in need of fertility preservation, especially those with no male partner or sperm donor.

Cryopreservation of embryos is the only treatment that is clinically established. This procedure is offered to females in a stable relationship or in situations where the female has arranged for a sperm donor.<sup>6</sup> It is a procedure which also, as for cryopreservation of oocytes, demands hormonal ovarian stimulation in advance for the possibility to harvest mature oocytes by using a transvaginal probe. After collection of oocytes and *in vitro* fertilization, the embryo can be frozen down and used later in situations of premature ovarian failure.

Cryopreservation of ovarian tissue is an option for prepubertal girls. It is possible to preserve whole ovarian tissue or just cortical strips, which both contain many primordial follicles. Removal of cortical strips and not removal of a whole ovary in pediatric patients is advocated due to uncertain outcome in the latter.<sup>1</sup> However, scientists disagree when it comes to preferable methods for removal of ovary tissue for cryopreservation. Both removal of whole ovary and removal of just cortical strips have resulted in live births after reimplantation.<sup>10</sup> It is a relatively new technique introduced about a decade ago, yet it has become a reliable procedure with great results. It is important to have in mind the risks involved going through the laparoscopic surgery for a young patient. The risk of complications is a lot higher in children compared to adolescents and adults regarding anaesthesia.<sup>11</sup> However, for children over three years old, the risk is relatively low and it further decreases with increasing age. The procedure basically involves the highest risk of complications in children under the age of 12 months. The state of health of the child also influences the risk of complications regarding the procedure. In cases of hematological-, respiratory-, infectious-, biochemical- and/or other disorders, one should address these prior to surgery.<sup>11</sup>

Several countries offer cryopreservation of ovarian tissue, but few have established definite criteria in terms of whom the procedure should be offered. Legal aspects attached to the procedure are still to be worked out. UK Royal College of Obstetricians and Gynecologists has established a set of criteria for evaluating patients for this procedure. The patient should be under 30 years old, no previous chemotherapy or radiotherapy (patients



aged less than 15 years old with previous low-risk chemotherapy should be considered), realistic chance of long-term survival, and high risk of treatment-induced immediate ovarian failure, informed consent from patient or from parents, negative HIV and hepatitis serology and no previous children.<sup>1</sup> A Nordic network on Fertility Preservation came into being in 2008, challenging many medical professionals from different disciplines, hospitals and Nordic countries, to improve the survival and wellbeing of children during treatment of cancer and simultaneously trying to protect the future fertility of these children. Collaboration is arranged with respect to define and improve fertility preservation routines, agree on routine and research areas and write a Nordic document of recommendations. Recommendations and guidelines both from the US and UK is used. In Norway the recommendations used today at OUS Rikshospitalet have an upper age limit of 35.<sup>12</sup> The treatment is solely reserved to women planned to undergo treatment that may cause premature menopause or infertility. The risk of infertility must be greater than 50%, due to the experimental nature of the treatment, and the risks involved. Reimplantation of ovarian tissue is not offered to patients suffering from generalized disease such as leukemia. However, cryopreservation may still be offered to these patients, due to the future possibility of *in vitro* maturation of oocytes. Information about the procedure and consent from the patient is necessary.

Reimplantation of the cryopreserved ovarian tissue can be done either by heterotopic or orthotopic transplantation. In orthotopic transplantation the transplant is replaced into its original position, whereas in heterotopic transplantation the cortical tissue is grafted subcutaneously at various sites, most commonly in the forearm or in the abdominal region. Heterotopic autotransplantation necessitates the use of *in vitro* fertilization, whereas spontaneous pregnancies are reported in patients that have gone through orthotopic transplantation. As illustrated in figure 2, orthotopic autotransplantation of ovarian tissue may be attached to the remaining ovaries, or sutured into subperitoneal pockets in close relation to the ovaries and the fimbrias of the fallopian tubes.<sup>13</sup>

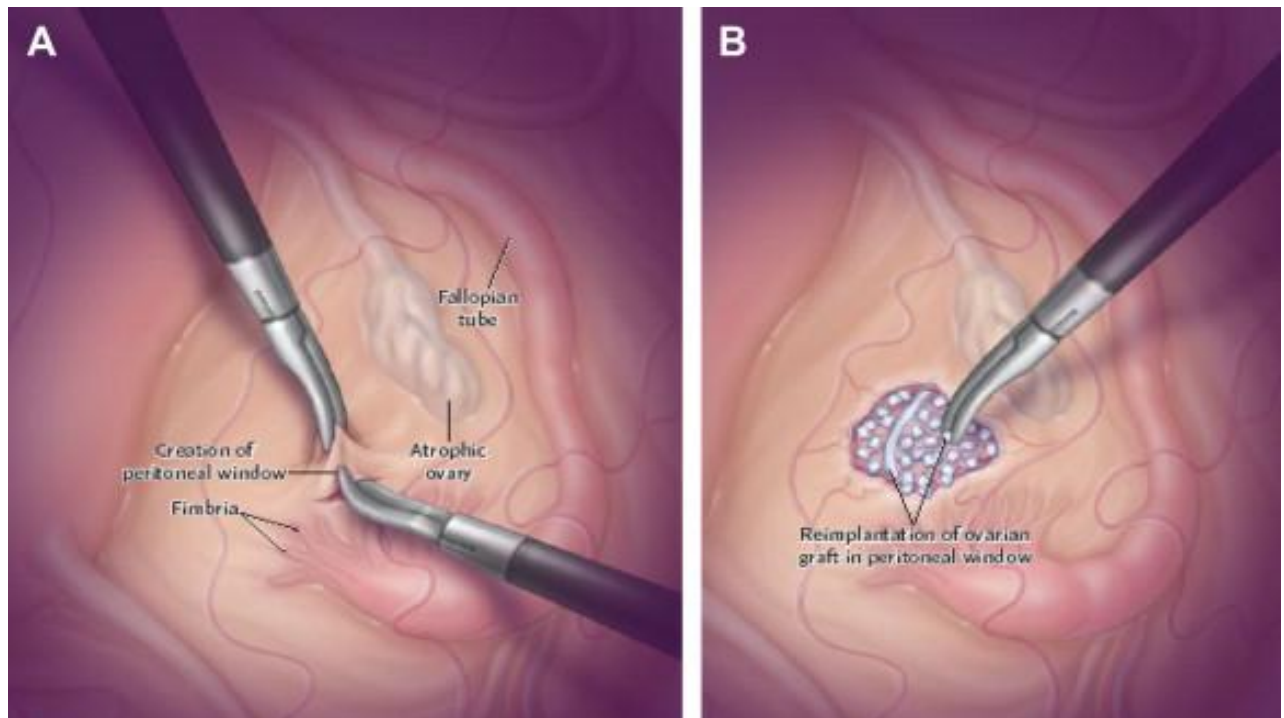


Figure 2 Orthotopic transplantation of a large strip and 35 small cubes of frozen-thawed ovarian tissue through a peritoneal window. The graft is placed in close relation to the fimbriae of the fallopian tube.<sup>14</sup>

## **Common malignant diseases in children**

1. CNS tumours. This group of tumours is the most common type of solid tumours in childhood cancer. The CNS tumours can be divided into intracerebral and intraspinal tumours; of which the latter contribute 10%. The most common CNS tumours in children are the gliomas (astrocytomas) (50%), medulloblastomas (20%), and ependynomas (10%).
  - The gliomas are divided into low-grade and high-grade gliomas. Low-grade and high-grade gliomas are further divided into several groups based on histology.<sup>15</sup>
  - Medulloblastoma is a primitive neuroectodermal tumour (PNET). WHO defines five variants of medulloblastoma, based on different histologic phenotypes.<sup>15</sup> The incidence of medulloblastoma is highest in the first decade of life. At the time of diagnosis 35% of the patients have metastasis to distant parts of the CNS. The prognosis depends on the age of the child and the stage of the disease.
  - Ependynomas contribute to 6-12% of intracranial tumours in children.

Concerning the low-grade gliomas the main treatment is complete surgical resection. When surgical resection succeeds, event free survival (EFS) exceeds 90%. Adjuvant chemotherapy is given in order to treat residual tumour tissue, and delay or maybe prevent the use of radiation therapy. The high-grade gliomas are treated by adjuvant chemotherapy followed by maximum resection. In addition to surgery and chemotherapy, treatment involves radiation to the tumour site and surrounding brain tissue. The survival rate for children with high-grade gliomas differs widely depending on the tumours histological feature, and to which extent all tumour tissue has been surgically removed. One study found EFS 45% at >90% tumour resection, and only 17% survival when tumour resection was below 90%. The most common late effects are impaired concentration, short time memory, and a gradually decrease in intelligence the first two years following radiation. Some children also experience endocrine disturbances, second neoplasms and hematologic malignancies. Standard treatment of medulloblastoma is maximal surgical resection of tumour masses, and

craniospinal radiation therapy (CSRT). Chemotherapy has improved the overall survival rate, using different protocols of multidrug chemotherapy. For the standard risk medulloblastoma, five year EFS above 80% is achieved in several studies.<sup>15</sup> The prognosis for children with metastatic medulloblastoma is poorer. Studies indicate an overall survival rate between 30-40% in this group.<sup>15</sup> However, recent studies have shown promising results using new chemotherapy regimens that include the use of cisplatin, etoposide, cyclophosphamid and vincristine, achieving 5-year EFS of 70%.<sup>15</sup> The main treatment of ependynoma is maximal surgical resection. Prognoses are uncertain, and different studies report 5-year EFS from 18-55%. Chemotherapy and radiation therapy have not been established as standard treatment.

## 2. Acute lymphocytic (ALL) - and myelogen leukemia (AML)

Acute lymphocytic leukemia (ALL) is characterized by abnormal proliferation (neoplasm) of precursor lymphoid cells in bone marrow, and constitutes for approximately 80 % of all leukemia, and approximately 25 % of the overall incidence of cancer in children. The WHO classifies ALL into three subgroups based on immunological phenotype of the disease.

- The most common subgroup often referred to as acute lymphoblastic leukemia, emanates from precursor B-cells lymphocytes and makes up about 80 % of the acute lymphocytic leukemia. The subgroup referred to as lymphoblastic lymphoma makes up about 10-15%.
- The Burkitts leukemia characterized by neoplasm of more mature B-cells, constitute less than 5 % of the cases.
- Acute myelogen leukemia (AML) is characterized by abnormal proliferation of precursor cells in the myeloid cell line. Malignant disease due to acute myelogen leukemia makes up about 15% of all leukemia in children. WHO classifies AML in subgroups (M0-M8) based on clinical presentation. In most cases the origin leukemia is not obtained, but Down syndrome, maternal exposure to pesticides and other DNA-damaging agents and infection by the John Cunningham virus are related to risk of developing the disease. Due to different immunological characteristics of the disease and the overall heterogeneity of leukemia, treatment must be outlined for each case.

However, there are several joint steps in treatment for acute lymphocytic leukemia in general. Medical treatment of ALL in the Nordic countries is outlined by Nordic Society for Pediatric Hematology and Oncology (NOPHO). It is separated into three successive phases of cytotoxic therapy named induction-, consolidation- maintenance phase. Induction phase involves a combination of cytostatics such as daunorubicine, vincristine, prednisolone, doxorubicin , cyclophosphamide, cytarabin, merkaptopurin and asparaginase and lasts for about a month.<sup>16</sup> Consolidation phase involves high dose of methotrexate, cyclophosphamide, cytarabin, dexametasone, vincristine, atracycline, asparaginase, cyclophosphamide, tioguanin and cytarabine. The last phase includes high doses of methotrexate and mercaptopurines, high doses of asparaginase followed by another induction phase. All children diagnosed with ALL are treated with intraspinal injections of methotrexate due to the high risk of having malignant cells in the central nervous system. Patients who are diagnosed with spread of malignant cells to the central nervous system, the cytotoxic treatment is further intensified and may also involve craniospinal radiation. The heterogeneous presentation of AML also demands individual follow up in terms of medical treatment. However, NOPHO has composed a set of joint steps in treatment in general, which includes intrathecal methotrexate, infusion of cytarabin and etoposid, and peroral tioguanin. Further treatment includes intravenous idarubicin, cytarabin and mitoksantron. In case of insufficient remission of blasts the treatment continues with fludarabin, cytarabin, G-CSF (granulocyte inducing factor) and daunorubicin (FLAG).

### 3. Lymphoma

Lymphoma is the third most common cancer seen in children and is characterized by a malign transformation of lymphocytes, diversified into Hodgkin- and non-Hodgkin lymphoma.

- Hodgkin lymphoma is further divided into a classical- and a nodular lymphocyte predominant Hodgkin lymphoma. The classical type encompasses nodular sclerosing-, mixed cellularity-, lymphocyte rich- and lymphocyte depleted lymphoma. Hodgkin lymphoma makes up about 30-40 % of the overall incident of lymphoma in children. The malignant transformation of lymphocytes usually involves the B-lymphocytes, and common lymphoid tissues affected are lymphatic nodes and spleen.

- Non-Hodgkin lymphoma makes up about 60% of the overall lymphoma in children. Non-Hodgkin lymphoma is divided into three subgroups named non-B-cell lymphoma, B-cell lymphoma and large cell anaplastic lymphoma. The cause of lymphoma is not known, but several factors have been connected to risk of developing the disease. Reduced immune system due to immunosuppressive treatment, HIV/AIDS, infection due to Epstein Barr virus, history of chemotherapeutical treatment and inheritance are listed as known risk factors of developing the disease.

The Ann Arbor staging system is used for both Hodgkin- and Non-Hodkin lymphoma. The first stage is characterized by involvement of one lymphatic node. The second stage involves disease in two or more lymphatic nodes on both sides of diaphragm. The third stage involves disease in a whole lymph region on both sides of diaphragm. The fourth stage is characterized by diffuse or disseminated disease in one or more extra lymphatic organ/tissue, with or without affection of lymphatic nodes.<sup>17 18</sup> Staging also further involves differentiation into subgroups A or B. The latter involves patients with affection of the general condition, while group A does not involve affection of the general condition.

The different classical Hodgkin lymphoma subgroups manifest differently in each patient, and treatment therefore has to be elucidated in each case. However, treatment involves a group of common used chemotherapeutic agents. A large part of treatment of Hodgkin lymphoma also comprises radiation therapy. In terms of Hodgkin lymphoma in children or adolescents under the age of 18, the majority of the patients receive radiation therapy. The initial treatment includes different chemotherapeutic agents. This is different compared to treatment in adults, whom always get radiation treatment before chemotherapeutic therapy. The protocol used for treatment of lymphoma in children is drawn up by the Gesellschaft für Pädiatrische Onkologie und Hämatologie in Germany. Treatment for females less than 18 years of age is OPPA (oncovin, procarbazine, prednisone and adriamycin), and in terms of more advanced disease (stadium IIB and higher) treatment includes COPP (cyclophosphamide, vincristine, procarbazine and prednisolone). From January 2008 a new protocol developed by EuroNet-Pediatric Hodgkin's Lymphoma Group has been used in terms of advanced disease (treatment of therapy group two and three).<sup>19</sup> Patients are divided into three treatment groups depending on extent of disease. The protocol is based on a randomized controlled study where one group gets COPP while the other gets

COPDAC (cyclophosphamide, dacarbazine, vincristine, procarbazine and prednisolone). The intention is to find out if dacarbazine, which reduces fertility, can be replaced by procarbazine.<sup>19</sup> The OPPA cure is therefore abandoned, and replaced with OEPA (doxorubicin, vincristine, etoposid and prednisolone) cure. Radiation is used if there is any malignant tissue left after the initial treatment.

Treatment of B-cell lymphoma is divided into seven blocks which encompasses chemotherapeutic agents such as dexametason, cyclophosphamide, metotrexate, cytarabine, prednisolone, vincristine, etoposide, ifosphamide, doxorubicin and vindisine. The overall treatment lasts for one to seven months. The treatment for the remaining non-Hodgkin lymphomas is the same as for Burkitts leukemia.

### Solid tumors outside CNS:

#### Neuroblastoma

Neuroblastoma is the most common solid tumour outside the CNS in Children.<sup>15</sup> The tumour emanates from the neural crest, and is often located in the adrenal medulla, or along the paraspinal sympathetic ganglia. Neuroblastoma is staged according to the INRG staging system:

Stage L1: Local resectable tumour. Stage L2: Localized tumour, with presence of IDRF (image defined risk factors.). Stage M: Metastatic tumours. Stage Ms: Metastases confined to the skin, liver, and/or bone marrow in children younger than 18 months of age.<sup>20</sup>

70% of the patients have metastatic disease at the time of diagnosis. The most common sites of metastatic spread are cortical bone, bone marrow, lymph nodes, liver and skin.

The low-risk patient is an infant less than one year of age with local disease. In such cases surgery alone achieves survival rates above 95%. In the intermediate risk group survival rates between 71-94% has been reported, after treatment with a combination of surgery and chemotherapy. The standard treatment includes use of cyclophosphamide and doxorubicin. In some cases treatment also encompasses cisplatin. The overall survival rate in the high-risk group is below 50%, in spite of combined treatment by surgery, radiation and chemotherapy. The backbone of this treatment includes cisplatin, cyclophosphamide and doxorubicin.

### Wilms' tumour

Wilms' tumour, or nephroblastoma, is the most common malignant renal tumour in children, and represents about 6% of overall incidence of cancer in childhood.<sup>15</sup> Most patients present with unilateral disease, but multifocal (12%) and bilateral (7%) lesions do also occur. The disease is staged from I-V.<sup>15</sup> The etiology is not entirely understood, but anomalies such as aniridia, hemihypertrophy and genital malformations are related to increased risk of developing Wilms' tumour.

Surgery is the main treatment of Wilms' tumour, but according to the SIOP 2001 protocol patients receive preoperative chemotherapy. Depending on the stage of the disease, adjuvant chemotherapy and/or radiation therapy may be given postoperative.<sup>21</sup>

SIOP 2001 protocol is standard chemotherapy regimen, although dosage and duration of the treatment depend on the histology and stage of the disease. Radiation therapy is used in advanced disease, and when the histology indicates a poor prognosis. The outcome is generally good, with a 4-year overall survival above 85%.<sup>15</sup>

### Soft tissue sarcomas

This group of malignant tumours emanates from mesenchymal tissues and is a heterogeneous group of tumours. The soft tissue sarcomas represent approximately 7% of all childhood malignancies. Although sarcomas may derive from all mesenchymal tissues such as connective tissue, fat cells, smooth muscle cells and blood vessels, the most common site of origin is striated muscle (rhabdomyosarcoma). The diagnosis of RMS is based on characteristics of striated muscle differentiation, combined with specific immuno-histochemical stains.<sup>15</sup> Analysis for detection of translocations and gene marker expression should also be done in order to get an accurate diagnosis and classification. RMS is classified into group I–IV.<sup>15</sup> In most cases no predisposing risk factors are known, but various syndromes and congenital anomalies increase the risk of rhabdomyosarcoma (RMS).

RMS is in general sensitive to chemotherapy and several agents such as vincristine, cyclophosphamide, dactinomycin, doxorubicin and ifosfamide are among the most effective agents.<sup>15</sup> The standard treatment is initially chemotherapy to reduce tumour size prior to surgery. Postoperative chemotherapy is mandatory and often in combination with radiation



therapy. Radiation therapy is often importunate in group II-III if complete resection is not possible with surgery or surrounding tissue contains malignant cells.

### Bone sarcomas

Sarcomas are classified in three different systems, IRS, TNM and MSTS.<sup>22</sup>

Osteosarcoma and Ewings sarcoma are the two most common primary malignant bone cancers in children.

In adolescents malignant bone tumours, i.e. Osteosarcomas, are the third most common cancer after leukemia and lymphoma. Peak incidence is during the growth spurt approximately at age 16 in girls, and 18 in boys. General characteristics of the disease are complex genetic alterations, including inactivation of the p53 and retinoblastoma genes. The histological appearance differs widely, but all osteosarcomas can proliferate and produce osteoid and/or bone tissue.

The Ewing sarcoma is the second most frequent malignant bone cancer after osteosarcoma. Most patients are diagnosed in second decade of life. Ewing sarcomas show great diversity when it comes to histological appearance and immunohistological features. The cause of is unknown. One theory is that the disease origin from pluripotent neuroectodermal stemcells, but conclusive evidence has not been found.<sup>15</sup>

Osteosarcoma which manifests as local disease is initially treated by surgery with complete resection and wide margins where possible. Postoperative chemotherapy is mandatory to treat micrometastasis that are present in most patients. Radiation therapy is done if surgery is insufficient to complete resection of malignant tissue.

Treatment of Ewing sarcoma is a multimodal therapeutic regimen which includes surgery, radiation therapy and chemotherapy. Overall survival rate is 50-65%. Preoperative radiation may be indicated in order to reduce tumour size and increase the possibilities to achieve complete resection. Various combinations of chemotherapy have been used.

## **Discussion**

### **Fertility preservation**

Females who have not entered puberty have limited options for fertility preserving procedures. Cryopreservation of ovarian tissue stands out as the only option for the time being. Regarding the procedure there are different guidelines in terms of preserving a whole ovary, or just cortical strips. Scientists work with the possibility of using *in vitro* maturation of follicles in fertility preserving treatment, but the procedure is still on an experimental stage. Females having entered puberty could choose other fertility preserving methods. When reached puberty, retrieval of mature oocytes is possible. However, going through weeks of hormonal ovary stimulation is seldom a desirable treatment for sick patients. The use of GnRH-agonist is not an option due to uncertain outcome, and cryopreservation of embryo demands a possible donor or a partner. Regarding the use of both chemo- and radiation therapy, the use of oophorectomy is seldom an option. The procedure can all in all be an alternative for a patient planned for radiation. However, there are scarce scientific results concerning this uncertain treatment. The protection of ovary function in adults having gone through oophorectomy is uncertain – less than 50 % have preserved ovary tissue.

The overall treatment for leukemia has drastically improved the last couple of decades. Going back 40 years the disease had a mortality rate of 100%. Today children with ALL have a survival rate ranged from 57-89% varying with different risk groups. In elucidation of better treatment and survival rate for ALL, the demand for better worked out methods for fertility preservation is importunate. The general treatment is known to be damaging to ovarian reserves, especially due to use of alkylating agents such as cyclophosphamide, which is highly toxic to ovarian tissue, and treatment with cytotoxic antibiotics such as doxorubicin which is rated as medium risk of developing premature ovarian failure.

Wallace et al emphasize that today's treatment of ALL in children and adolescents has less than 20% chance of inducing subfertility after treatment.<sup>1</sup> Emphasis is placed on the unknown cause of combination chemotherapy, and therefore it is difficult to estimate the exact risk of ovarian damage in elucidation of chemotherapy. It is also known that general cytotoxic treatment induces the velocity of follicle depletion, and thereby elevates risk of premature menopause. In terms of chemotherapy conditioning for bone marrow transplantation the risk of developing premature ovarian failure is more than 80% .

Despite exquisite methods for preserving ovarian tissue in patients with acute leukemia, there are still several challenges related to reimplantation of cryopreserved ovarian tissue. A study carried out by Dolmans et al demonstrated the potential risk of transferring malignant cells in cases of reimplantation of ovarian tissue.<sup>23</sup> The study revealed that histology examination of ovarian tissue is not enough to exclude malignant cells in the transplant. By using quantitative reverse-transcribed polymerase chain reaction (RT-PCR) and six months xenografting to immunodeficient mice, the study revealed the unfortunate possibility of reintroducing malignant cells to a patient in situations of reimplantation of cryopreserved tissue. The study illustrates the absolute need of additional studies on this topic, before patients recovered from acute leukemia get ovarian tissue reimplanted.

Chemotherapeutic agents as mentioned above in treatment for non-Hodgkin- and Hodgkin lymphoma include most of the same chemotherapeutic agents used in treatment of acute leukemia in general. The ovarian toxicity of the treatment is therefore known. The COPP treatment leads to ovarian damage in a relatively high percentage of the cases. In terms of Hodgkin lymphoma, treatment usually includes radiation, which toxicity relates to dose and localization. Total body radiation prior to stem cell transplantation causes ovarian failure in most of the patients. For the latter there has been discussed risk of premature birth, spontaneous abortion, reduced weight and length of the uterus because of damaged vascularisation of the ovaries. However, there is no documentation on elevated risk of spontaneous abortion or fetal malformation after radiation if conception occurs more than a year after cessation of the treatment.<sup>8</sup> Despite the high toxicity of treatment, survival rates are high. With over 90 % survival rates in patients with Hodgkin lymphoma and over 80 % survival rates in patients with non-Hodgkin lymphoma, this makes the need for adequate methods for fertility preservation importunate as in cases of acute leukemia in children and adolescents.

The use of cryopreservation is often chosen in terms of lymphoma, probably because the risk of reintroducing malignant cells is relatively low compared to patients with leukemia, and therefore it is the most preferable method for fertility preservation.<sup>24</sup> Before transplantation of ovary tissue, the use of histological examination is performed to exclude the risk of reintroducing malignant cells. Scientists focus on the insufficient use of histological examination of the tissue before transplantation considering the risk of reintroducing malignant cells. A case report which evaluated ovaries from a young female with Hodgkin

lymphoma demonstrated the risk of reintroducing malignant cells.<sup>24</sup> Therefore, despite general knowledge that Hodgkin lymphoma seldom infiltrates ovary tissue, that particular case report calls attention to the need of exquisite procedures to exclude the risk of reintroducing malignant cells when using cryopreservation as method for fertility preservation. The study done by Dolmans et al also emphasizes that histological examination is not enough to exclude malignant cells in ovary tissue transplant. Patients with Hodgkin lymphoma constitute the majority of patients who have received reimplantation of cryopreserved ovarian tissue.<sup>24</sup>

Due to serious adverse effects like growth retardation, damage to organs, and risk of other radiation induced malignant diseases; radiation therapy is carried out only in situations of absolute necessity. However, the use of radiation is often necessary, and in terms of stem cell transplantation total body radiation is essential.

Regarding females who have reached puberty, there are other fertility preserving methods such as retrieval of mature oocytes. However, that is just an option in theory. The time needed for ovarian stimulation is seldom an option for patients with lymphoma, especially not for patients with B-cell lymphoma, due to the need of quick initial treatment.

The main treatment for CNS tumours is surgery, which has no impact on ovarian function or increased risk for developing ovarian failure. In glioblastomas and ependynomas chemotherapy is used only as adjuvant therapy and in relatively small doses. Hence, the risk of developing ovarian failure is only slightly increased. Young children with medulloblastoma are treated with surgery and intensive chemotherapy including cyclophosphamide and cisplatin. The risk of developing premature ovarian failure is high. Radiation therapy is widely used in order to treat CNS malignancies. The risk of direct impact on the ovaries is quite small considering the distance from the field of radiation to the ovaries. However, cranial radiation may cause damage to the hypothalamic-pituitary axis and cause amenorrhea or delayed menarche.

The chemotherapeutic treatment of neuroblastoma varies with the stage of the disease and to which extent surgery leads to complete resection. The standard regimen includes cyclophosphamide which is an alkylating agent highly toxic to ovarian tissue. Cisplatin and doxorubicin are also part of the general treatment and are rated as intermediate risk agents for developing ovarian failure. The treatment is considered as high risk for developing premature

ovarian failure. About 95% of neuroblastomas are diagnosed in children under the age of 5, and only a few cases occur after menarche. Median age at the diagnosis is 22 months. The options for fertility preserving therapy are therefore reduced to cryopreservation of ovarian tissue. Even though the use of cryopreservation of ovary tissue does not conflict with cancer treatment, the procedure which includes general anaesthesia could by itself pose a high risk for complications in females less than 12 months of age.

In cases of complete resection of unilateral disease caused by Wilms' tumour, the risk of premature ovarian failure drops significantly when treatment with chemotherapeutic agents is successful. When manifested advanced disease the protocol involves use of cyclophosphamide. Wilms' tumour responds to radiotherapy which is widely used in terms of advanced disease. The field of radiation includes the abdominal midline and columnae in order to achieve adequate treatment. Nevertheless, scattered radiation may cause destruction of ovarian tissue and the risk of ovarian failure must be considered.

General treatment of soft tissue sarcomas and malignant bone tumours include use of highly ovarian toxic chemotherapeutic agents and radiation therapy. The risk of premature ovarian failure related to this treatment is high. However, in terms of malignant bone tumours detected at an early stage of the disease surgery may be curative.

## **Conclusion**

Due to the high survival rates and good prognosis in patients with malignancies, the need for safe and effective fertility preserving methods are necessary. Despite the several techniques offered for females in need of fertility preserving methods, few are available for prepubertal females. The only option for female not reached puberty is cryopreservation of ovarian tissue. The need for further examination and studies regarding safety of the procedure are also importunate. Use of fertility preserving methods such as cryopreservation of ovarian tissue is very new, and few guidelines have been made regarding who should and who should not be offered this treatment.

The need of better worked out procedures to exclude the risk of reintroducing malignant cells regarding transplantation of cryopreserved ovary tissue is importunate. The use of *in vitro* maturation (IVM) belongs to the future for the time being, but will then be a procedure for fertility preservation in prepubertal females. By bypassing the retransplantation of ovary tissue and mature follicles *in vitro*, the risk of reintroducing malignant cells is

avoided. A study using quantitative reverse-transcribed PCR (RT-PCR) has also shown promising results in order to prevent the reintroduction of malignant cells. A lot of studies encompass growth factors and oocyte development. The role of anti-müllerian hormone (AMH) on follicle development is known, but further studies have to be implemented to elucidate the effect of using AMH in fertility preserving treatment.

For the time being there are no restrictions to whom the procedure could be offered. However, there are restrictions concerning retransplantation of cryopreserved tissue to patients recovered from leukemia, due to high risk of reintroducing malignant disease. Wallace et al recommend that ovarian tissue cryopreservation should only be considered for young women at high risk of premature menopause.<sup>1</sup> However, that is in conflict with the knowledge that all fertile females who have gone through chemotherapeutic treatment have reduced fertility – despite normal menstruation after treatment. It is also important to recognize the risk of premature ovarian failure due to the disease itself.

Numerical factors should be taking in to account before going through with the procedure. The risk of complications concerning anaesthesia and laparoscopic surgery are low. However, it is important to have in mind the increased risk of complications in children under the age of three, especially under the age of twelve months, and children with serious hematological-, respiratory-, infectious-, biochemical- and/or other disorders. Information concerning fertility preserving methods and the various risks and limitations of the treatment should be given to the patient and parents at an early stage. That is to ensure that the choice of going through with the procedure is well informed, and that fertility preservation does not delay the treatment of the malignant disease.

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